

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

1-33. Canceled

34. (currently amended) An *in vitro* assay method for detecting steroid hormone-like cancer cell growth stimulation by a substance of interest, the method comprising:

maintaining a predetermined population of steroid hormone-responsive cells in a steroid hormone-free nutrient medium comprising a basal nutrient fluid substantially devoid of unbound Fe (III) and comprising calcium ions and an amount of isolated secreted immunoglobulins that inhibits steroid hormones sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said cells also being steroid hormone dependent for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and medium to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions;

determining the cell population in said test mixture after said predetermined period of time, a measurable increase in said cell population indicating a steroid hormone-dependent like cell growth stimulating effect by said substance of interest.

35. (Original) The assay method of claim 34 comprising maintaining serum-free assay conditions.

36. (Original) The assay method of claim 34 comprising adding steroid-hormone depleted serum to said nutrient medium.

37. (currently amended) The assay method of claim 34 further comprising the step of obtaining adding non-heat inactivated serum containing said immunoglobulin inhibitors.

38. (previously presented) The assay method of claim 34 wherein said immunoglobulin inhibitor comprises at least one secretory immunoglobulin chosen from the group consisting of dimeric/polymeric IgA, polymeric IgM and IgG.

39. (Original) The assay method of claim 38 wherein at least one secretory immunoglobulin is chosen from the group consisting of IgG1 and IgG2.

40. (Original) The assay method of claim 39 wherein at least one secretory immunoglobulin is IgG1 κ .

41. (Currently Amended) The assay method of claim 34 wherein said substance of interest contains or is suspected of containing proteolytic activity, the method further comprising selecting an immunoglobulin inhibitor of a steroid hormone that resists protease degradation.

42. (Original) The assay method of claim 34 wherein said selected immunoglobulin inhibitor comprises IgA2.

43. (Original) The assay method of claim 34 further comprising:

maintaining a second predetermined population of steroid hormone-responsive cancer cells in a steroid hormone-free nutrient medium comprising a quantity of inactivated immunoglobulin inhibitor that is incapable of inhibiting cell growth, said cells also being steroid hormone responsive for proliferation *in vivo* when implanted into a suitable host;

adding said substance of interest to said cells and medium, to yield a test mixture;

incubating said test mixture for a predetermined period of time under cell growth promoting conditions;

determining the cell population in said test mixture after said predetermined period of time, a measurable increase in said cell population indicating a control level cell growth stimulating effect by said substance of interest in the presence of said quantity of inactivated immunoglobulin inhibitor.

44. (currently amended) A method of detecting a steroid hormone antagonistic substance comprising:

maintaining a predetermined population of steroid hormone responsive cancer cells in a nutrient medium comprising a basal nutrient fluid ~~substantially~~ devoid of unbound Fe (III) and comprising calcium ions and comprising a quantity of an immunoglobulin inhibitor of a steroid hormone comprising secreted immunoglobulins sufficient to inhibit cell growth in the absence of an inhibition-reversing amount of said steroid hormone, said cells also being steroid hormone responsive for *in vivo* proliferation;

adding a defined amount of said substance of interest to said cells and medium;

adding to said cells and medium a defined amount of steroid hormone sufficient to stimulate cell growth in the presence of said inhibitor and in the absence of said substance of interest, to yield a test culture;

incubating said test culture for a predetermined period of time under cell growth promoting conditions;

testing said substance of interest for cytotoxic effects on said cells; and

determining the cell population in said test culture after said predetermined period of time, a lack of measurable increase in said cell population not attributable to cytotoxic effects of said substance indicating a steroid hormone antagonistic effect by said substance of interest.

45-84 Cancelled

85. (Original) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of GH₁, GH₃ and GH₄C₁ rat pituitary tumor cells, and said medium comprises 100 ng/mL to 10 μ g/mL insulin, 0.3 - 10 nM triiodothyronine, 2 - 50 μ g/mL

diferric transferrin, 5 - 100 μ M ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, and 2 - 10 μ M deferoxamine.

86. (Withdrawn) The *in vitro* cell culture model of claim 75 wherein said steroid hormone-responsive culture cells are chosen from the group consisting of GH₁, GH₃ and GH_{4C1} rat pituitary tumor cells, and said medium comprises 10 ug/mL insulin, 1 nM triiodothyronine, 10 μ g/mL diferric transferrin, 10 μ M ethanolamine, 500 μ g/mL bovine serum albumin (BSA), 10 ng/mL selenium, and 10 μ M deferoxamine.

87-94. (Canceled)

95. (Currently amended) The method of claim 34 comprising:

maintaining a predetermined population of estrogen responsive cancer cells in a steroid hormone-free nutrient medium comprising a quantity of immunoglobulin inhibitor of a steroid hormone sufficient to inhibit cancer cell growth in the absence of an inhibition-reversing amount of estrogen, said cells also being estrogen responsive for proliferation *in vivo* when implanted into a suitable host;

adding a defined amount of said substance of interest to said cells and medium, to yield a test culture;

incubating said test culture for a predetermined period of time under cell growth promoting conditions; and

determining the cell population in said test culture after said predetermined period of time, a measurable increase in said cell population indicating an estrogen-dependent like cell growth stimulating effect by said substance of interest, whereby an estrogenic substance is detected.

96. (Original) The method of claim 95 further comprising testing said substance of interest for binding to estrogen receptor gamma.

97. (Original) The method of claim 95 further comprising testing said substance of interest for cytotoxic effects on said cells.

98. (Original) The method of claim 95 further comprising selecting estrogen responsive cancer cells containing estrogen receptor gamma.

99 - 108. (Canceled)

109. (Original) The method of claim 34 wherein said steroid hormone-free nutrient medium comprises no more than about 1 μ M unbound Fe(III).

110. (Original) The method of claim 34 wherein said medium comprises a Fe (III) chelating agent.

111. (Original) The method of claim 34 wherein said medium comprises a cell attachment promoting protein.

112. (Original) The method of claim 34 wherein said medium contains about 1-50 mM calcium ion.

113. (Original) The method of claim 34 wherein said basal nutrient fluid comprises D-MEM/F-12.

114. (Original) The method of claim 34 wherein said medium comprises 100 ng/mL to 10 μ g/mL insulin, 0.3 - 10 nM triiodothyronine, 2 - 50 μ g/mL diferric transferrin, 5 - 100 μ M ethanolamine, 0.2 - 5.0 mg/mL bovine serum albumin (BSA), 5 - 20 ng/mL selenium, 2 - 10 μ M deferoxamine, and, optionally, at least one component chosen from the group consisting of 1 - 50 ng/mL EGF, 0.2 - 20 ng/mL aFGF, 5 - 50 μ M phosphoethanolamine, 50 - 500 μ g/mL linoleic acid-BSA, 1 - 50 μ g/mL reduced glutathione, 0.5 - 2.0 mM glutamine, 1 - 10 μ g/mL heparin, and 20 - 50 μ g (per 35-mm diameter culture dish) human fibronectin.

115. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium comprises no more than about 1 μ M unbound Fe(III).

116. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium comprises a Fe (III) chelating agent.

117. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium comprises a cell attachment promoting protein.

118. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium contains about 1-50 mM calcium ion.

119. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium is serum-free.

120. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium comprises steroid-hormone depleted serum.

121. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium comprises D-MEM/F-12.

122. (currently amended) The method of claim [[44]] 34, wherein said nutrient medium comprises:

100 ng/mL to 10 μ g/mL insulin,
0.3 - 10 nM triiodothyronine,
2 - 50 μ g/mL diferric transferrin,
5 - 100 μ M ethanolamine,
0.2 - 5.0 mg/mL bovine serum albumin (BSA),
5 - 20 ng/mL selenium,
2 - 10 μ M deferoxamine, and,
optionally, at least one component chosen from the group consisting of
1 - 50 ng/mL EGF,
0.2 - 20 ng/mL aFGF,
5 - 50 μ M phosphoethanolamine,
50 - 500 μ g/mL linoleic acid-BSA,
1 - 50 μ g/mL reduced glutathione,
0.5 - 2.0 mM glutamine,
1 - 10 μ g/mL heparin, and
20 - 50 μ g human fibronectin (per 35-mm diameter culture dish).